Analytical Supercritical Fluid Chromatography and Extraction

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CHAPTER 5

ANALYTICAL SUPERCRITICAL FLUID EXTRACTION

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5.1 Introduction

Supercritical fluid extraction (SFE) is a relatively new technique in the field of analytical chemistry, having evolved in the last decade as an alternative method of preparing samples prior to analysis. SFE offers many advantages to the analyst that are not inherent in other sample preparation techniques, such as distillation, extraction with liquid solvents, or low resolution liquid chromatography. The most unique property of supercritical fluids for extraction purposes is the ability to adjust their "solubilizing power" primarily *via* mechanical compression (and additionally *via* temperature), thereby providing the possibility of using one supercritical fluid to extract a host of analytes of varying polarity and molecular size. In addition, solute-fluid binary diffusion coefficients are much greater in supercritical fluid media than in liquid-liquid systems, thereby facilitating fast extraction from a variety of sample matrices.

The proper choice of supercritical fluid can also provide specific advantages when applied in sample workup prior to analysis. For example, the low critical temperature of supercritical CO_2 makes it an excellent candidate for extracting thermally labile compounds under conditions slightly above room temperature. In addition, CO_2 provides an extraction environment free from molecular oxygen, thereby limiting potential oxidation of the extracted solutes. Supercritical CO_2, unlike many liquid extraction solvents, is a nontoxic extraction medium; hence, its use in a laboratory environment can eliminate the cost and problems associated with solvent disposal as well as long term exposure of laboratory personnel to potential toxic vapors.

In practice, SFE can provide an appreciable savings in time and cost associated with sample preparation. As will be shown later in this chapter, on-line coupling of SFE to microcolumn chromatographic instrumentation permits the extraction and characterization of very small samples. In general, large polar compounds exhibit almost no solubility in supercritical CO_2,[1] making it an excellent extraction medium for the separation of nonpolar to moderately polar solutes from such matrices as inorganic solids. However, the solubility of polar analytes can be enhanced in many supercritical fluids by the addition of co-solvents, or modifiers,[2] at low levels to the dense gaseous phase.

By far, the most widely used extraction fluid has been supercritical CO_2; however, the extractability of polar solutes can be improved by using a more polar supercritical fluid. Table 3.1 is a tabulation of various supercritical fluid media and their associated critical properties which have been used in performing supercritical fluid extractions as well as SFC. Many of the listed fluids would not be suitable for practical extractions due to their unfavorable physical properties, costs, or reactivities. For example, ethylene which exhibits a subambient critical temperature has been widely investigated in the laboratory as an extractant. However, its flammability limits its application in many analytical problems. Conversely, most polar fluids have high critical temperatures which can prove destructive to both the analyte and extraction system. The isoelectronic analogue of CO_2, N_2O, has been shown to be a useful extracting fluid;[3] however, it exhibits a high reactivity toward many compounds and can cause dangerous physiological effects. Other fluids, like fluoroform (HCF_3), are unique in their ability to solubilize basic solutes through intermolecular hydrogen bonding in the supercritical fluid state,[4] but the exorbitant cost of the fluid limits its use for SFE.

It is useful to compare the physical properties exhibited by CO_2 under SFE conditions to those associated with liquid solvents under ambient conditions in order to gain a better understanding of the advantages which are attendant to conducting extractions in the supercritical fluid state. Table 5.1 compares the physical properties of CO_2 under typical SFE conditions (200 atm and 55°C) with parameters calculated for three liquid solvents: *n*-hexane, methylene chloride, and methanol at ambient conditions. The density of CO_2 at the above conditions is greater than the corresponding value for *n*-hexane, but lower than the densities exhibited by methanol or methylene chloride. Although density is only an approximate measure of intermolecular attraction, the value for CO_2 suggests that

Table 5.1. Comparison of Physical Properties of Supercritical CO_2 with Liquid Solvents at 25°C

	CO_2[a]	*n*-Hexane	Methylene chloride	Methanol
Density ($g\ mL^{-1}$)	0.746	0.660	1.326	0.791
Kinematic viscosity ($m^2\ s^{-1} \times 10^7$)	1.00	4.45	3.09	6.91
Diffusivity of benzoic acid ($m^2\ s^{-1} \times 10^9$)	6.0	4.0	2.9	1.8
$\left(\frac{P_{v,sat}\ solvent}{P_{v,sat}\ solute}\right)^b$	1.4×10^5	4.2×10^2	1.2×10^3	3.6×10^2

[a]At 200 atm and 55°C
[b]Solute is phenol at 25°C

near liquid-like densities can be achieved for this gas in its supercritical fluid state.

Likewise, kinetic-based properties such as viscosity and solute diffusivity, for CO_2, have values that are more typical of gases than those of the liquid state. These gas-like transport parameters contribute to improved rates of mass transfer for solutes in supercritical fluid media, resulting in faster extraction. The ratio of the saturated vapor pressures of the extraction solvents to that exhibited by a typical solute, phenol, at 25°C are also tabulated in Table 5.1. The vapor pressure for the CO_2/phenol case is 2-3 orders of magnitude larger than the corresponding ratios for the liquid solvent/phenol pairs. This accounts for the ease by which the dissolved solute (phenol) can be separated from CO_2 upon decompression, a phenomenon which is in stark contrast to the miscible liquid solute-solvent systems.

The intermediate properties exhibited by supercritical fluids permit the interfacing of SFE with GC, SFC, LC, and MS. Details of tandem arrangements of SFE with GC and SFC are presented in Sections 5.4 and 5.5 of this chapter; however, in such systems in

general, the supercritical fluid is delivered by a pump into an extraction cell where the extraction takes place under controlled pressure and temperature conditions. The extract is then swept into an injection system where it can be transferred to the eluent stream of the chromatographic system. Separation of the extract components takes place on the chromatographic column with subsequent detection by a general or selective method. The coupling of SFE with a suitable chromatographic method can also impart an extra degree of selectivity into the overall separation problem. Hence, by varying the extraction pressure and temperature in the cell, the analyst can change the composition of the extract and achieve additional fractionation of the sample. In addition, SFE may provide a solvent-free injection method for open tubular column chromatography where injection volume otherwise limits the minimum detectable level of trace solutes.

5.2 Fundamental Thermodynamic and Kinetic Parameters

The effective utilization of SFE in analytical chemistry requires an appreciation of the fundamental thermodynamic and kinetic parameters which impact on the distribution of the analyte between the dense fluid phase and the substrate that is being extracted. Reviews of the phase behavior[5] and thermodynamics[6] pertaining to supercritical fluid systems exist; however, similar treatises on the kinetics and mass transfer of solutes in supercritical fluids are limited. A thorough discussion of all theories and experimental studies describing supercritical fluid phase equilibrium is beyond the scope of this analytical text; consequently, this section will describe the key physicochemical factors which the analyst should know for utilizing SFE.

Four properties are seminal to planning and executing successful analytical SFE. These are the detectable "threshold pressure," the appropriate conditions for fractionating solutes, the occurrence of solubility maxima in supercritical fluid systems, and when possible, knowledge of the physical properties of the extracted solutes. The term "threshold pressure" was first mentioned by Giddings[7] to define the pressure at which the solute partitioned into the supercritical fluid. Unfortunately, such a parameter is dependent on the measurement technique employed;[8] however, even within this context it is a useful concept. For example, if one is trying to extract

a specific analyte from a matrix containing coextractable material, selective isolation of the target analyte may be possible if its threshold pressure is sufficiently removed from those of the coextractants.

The threshold pressure should not be confused with the pressure region where a significant increase occurs in the solubility of the solute in the supercritical fluid. This difference can be seen in Figure 5.1 where the solubility of naphthalene in CO_2 is plotted as a function of pressure. Here, the threshold pressure would appear to occur at values under 75 atm, depending on the experimental method used for its determination. The onset of the maximum differential solubility change appears to occur above 90 atm and parallels the increase in fluid density with pressure, a reflection of the increased molecular interaction between the solute and the solvent. The prediction of the maximum solubility change with pressure has been theoretically treated by Gitterman and Procaccia.[9]

Fractionation ranges for SFE can be theoretically estimated[10] or determined empirically by experimental methods. In general, the fractionation range potentially exists between the threshold pressure region of the solutes and the occurrence of solubility maxima for the dissolved solutes in the supercritical fluid. The fractionation of complex mixtures by SFE is frequently difficult unless appreciable differences exist in the molecular sizes, polarities, or volatilities of the mixture components. Enrichment of certain solute fractions can be affected by gradually increasing the pressure of the extracting fluid; however, this result is countered by a time-based fractionation effect. Enhancement of the SFE fractionation can be achieved by employing a thermal gradient or a chromatographic column downstream from the extraction module.

Solubility maxima occur for many solute types in supercritical fluid solvents.[11] Normally, the pressures associated with this phenomenon are quite high and thus would appear to be of little consequence to the analytical chemist. However, it has been recently demonstrated that the bulk removal of lipid phases by supercritical CO_2 is best effected at high pressures where lipid solubility is maximized.[12] Further increases in extraction fluid pressure can actually result in a "salting out" of the solute from the dense fluid phase as repulsive solute-solvent forces begin to predominate in the single phase system.

Although, not always available prior to SFE, a knowledge of the physical properties of the solute(s) can be of considerable aid in establishing optimal conditions for conducting the extraction. When extracting solid substrates, a knowledge of the solute or mixture

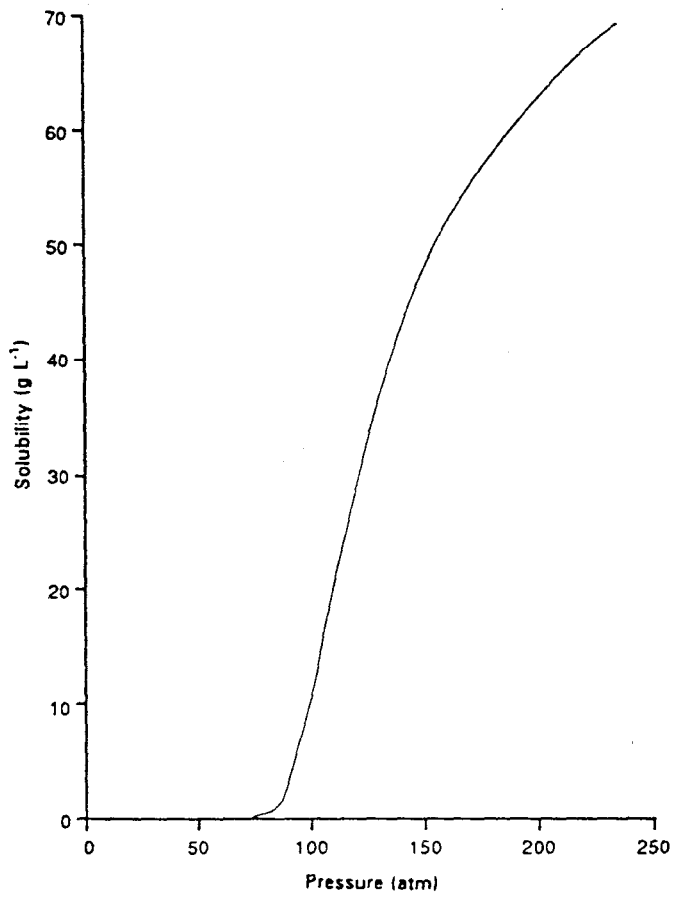

Figure 5.1. Solubility as a function of pressure for naphthalene in supercritical CO_2 at 45°C.

melting point is critical. In general, supercritical fluids are more effective extracting agents when the extraction is performed at a temperature above the melting point of the substrate. In this case, both mass transfer of the solute into the supercritical fluid is improved as well as solute solubility due to the weakening of the cohesive forces of the solid. Likewise, knowledge of the vapor pressure of the solute as a function of temperature can have a

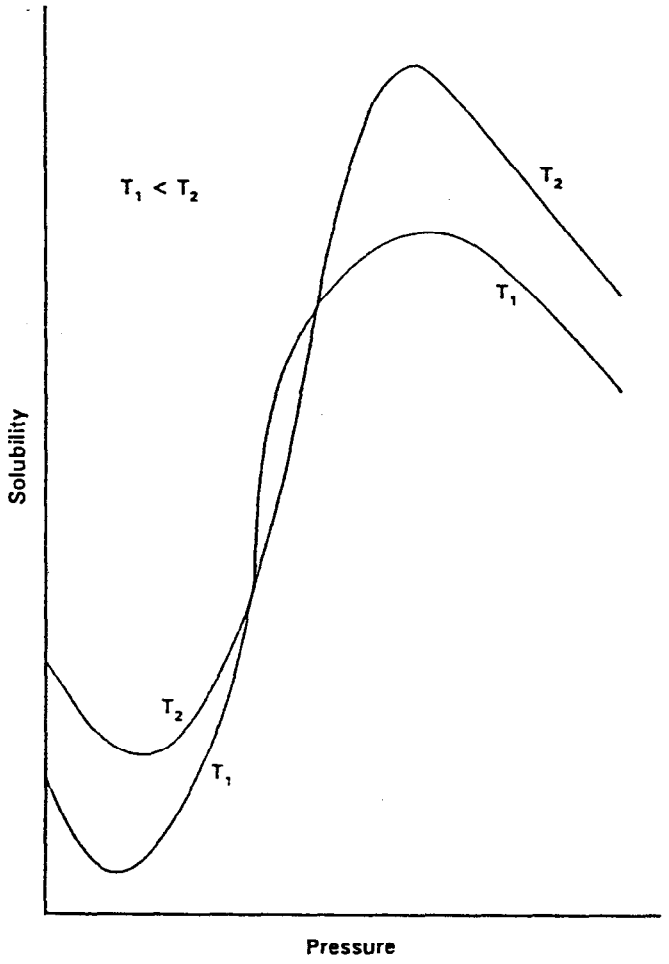

Figure 5.2. Generalized solubility isotherms as a function of pressure.

profound effect on both the recorded solubility and the separation factors that are obtained in multi-component solute separation schemes.[13]

The effect of varying both the pressure and temperature on the solubility of a solute during SFE is depicted in Figure 5.2. The recorded initial solubility in the noncompressed gas phase is a

function of the vapor pressure of the solute; however, upon additional compression of the supercritical fluid phase, a solubility minimum is observed. After the occurrence of the solubility minimum, there is an exponential rise in the solubility of the solute with increasing gas pressure, and a solubility maximum is eventually attained at a pressure which is determined by the extraction temperature. Note that the effect of increasing temperature in this case results in an increase in solubility at both low and high pressures; however, at intermediate pressures, the reverse trend may be observed. The latter region has been termed the "cross-over region,"[14] and its occurrence permits selective fractionation of solutes into the supercritical fluid medium.

Solubility trends in supercritical fluids. Today, there exists a substantial data base from which general rules regarding solute solubilities in supercritical fluids can be formulated. For the analyst employing SFE, the dependence of solubility on solute structure is, perhaps, the most important factor for predicting the relative effectiveness of SFE as a pre-analysis sample preparation method. In addition, an understanding of the importance of the absolute solubility of the solute is critical, since this will affect the time required for executing a particular extraction.

By far, the most extensive solubility data collected for solutes in a particular supercritical fluid are for binary CO_2/solute systems. Solubility trends in these systems suggest that nonpolar, lipophilic solutes exhibit the largest solubilities in supercritical CO_2, and the introduction of polar substituents into the molecular structure adversely affects the solubility of a compound in CO_2. Stahl,[15] on the basis of his extensive SFE/TLC studies, formulated generalized extraction rules which proved applicable to cases involving relatively low molecular mass solutes. These experimental observations are as follows:

(a) Hydrocarbons and other typically lipophilic organic compounds of relatively low polarity (*e.g.*, esters, ethers, lactones, and epoxides) can be extracted in the lower pressure range (*i.e.*, 70-100 atm).

(b) The introduction of strongly polar functional groups (*e.g.*, -OH and -COOH) makes the extraction more difficult. For the benzene derivatives, substances with three phenolic hydroxyls are still capable of extraction, as are compounds with one carboxyl and two hydroxyl groups. Phenols that cannot be extracted are those with one carboxyl and three or more hydroxyl groups.

(c) More strongly polar substances (*e.g.*, sugars and amino acids) cannot be extracted in the range up to 400 atm.

(d) Fractionation occurs in the pressure gradient when there are sufficient differences in the commencement of boiling or sublimation, *i.e.*, in the volatility and/or polarity, of the substances. The fractionation effects are most marked in the range where there is a sharp rise in the density and dielectric constant of CO_2.

Qualitative trends in solute solubilities have also been summarized by Hyatt[16] and Dandge,[17] and they have been quantitatively correlated with the aid of the reduced solubility parameter concept by King.[18] These correlations suggest that an increase in the molecular mass of a homologous or oligomeric series of solutes leads to a decrease in solubility in the supercritical fluid phase. An exception to this trend has been documented by Schultz and Randall[19] for the case of homologues partitioning between water and liquefied CO_2. Here, the addition of methylene groups accentuates the partitioning of the solute from the aqueous medium into the nonpolar phase, despite an increase in solute molecular mass. This trend has also been verified for solute partition into supercritical CO_2 from polar solvents.

It should be appreciated that high solute solubility in the extraction fluid is not always a prerequisite for applying SFE for analytical purposes. To illustrate this point, the solubility of the pesticide, alachlor, has been plotted (see Figure 5.3) over the pressure range of 50-300 atm. Note that a solubility in excess of 10 weight percent can be achieved in CO_2 at a compression level of approximately 270 atm. Such a solubility level is of critical importance in engineering applications of SFE where extractions are conducted for profit. However, for the analyst faced with extracting a 1-ppm level of alachlor from an environmental sample, the pressure requirements are not so stringent, and a rapid extraction can be achieved at much lower pressures despite the substantial reduction in alachlor solubility.

The use of co-solvents can also have a profound effect on increasing the solubility levels of polar solutes in supercritical fluids. For example, Wong and Johnston[20] have shown that the addition of 3.5 mol% of methanol to CO_2 at a pressure of 150 atm will increase the solubility of cholesterol seven-fold over that achieved using pure CO_2. In general, the addition of an entrainer to a supercritical fluid will enhance the solubility of a solute in the fluid phase as well as alter the separation factor between coextracted solutes.[21] Kurnik and Reid[22] have also shown that the presence of a coextracted solute can dramatically change the solubility level of a compound over that recorded for the simple binary system consisting only of the extracting fluid and the dissolved compound.

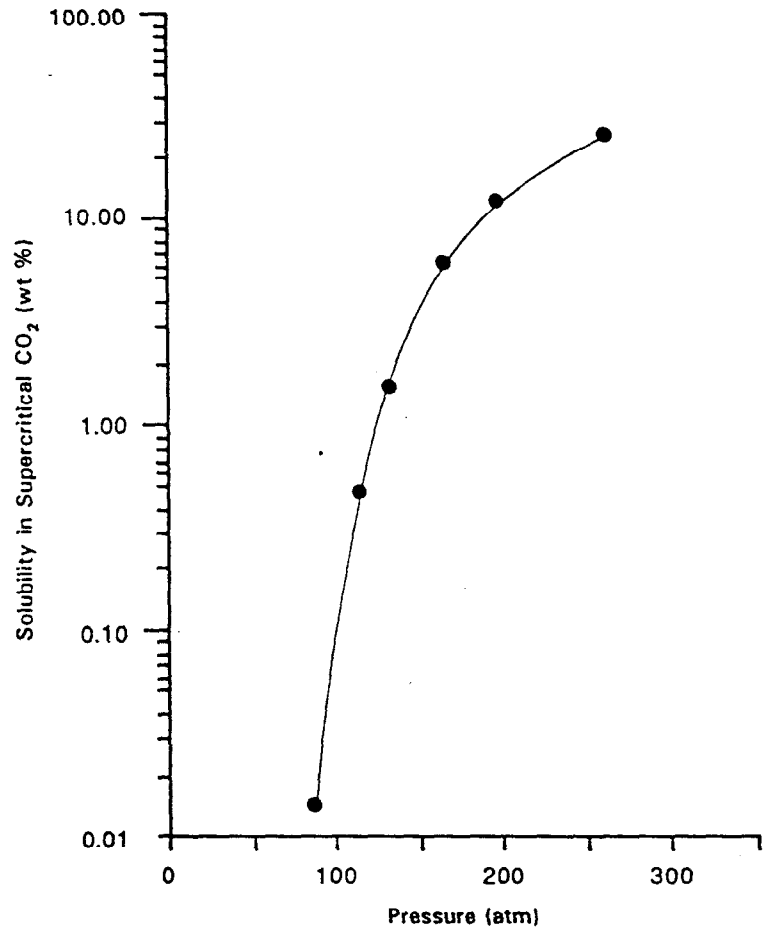

Figure 5.3. Solubility as a function of pressure for alachlor in supercritical CO_2 at 55°C.

Solubility theory. There have been few attempts to date to apply theory for the optimization of extraction conditions for analytical SFE or for the prediction of analyte solubilities in supercritical fluids. This is not surprising considering the relative immaturity of analytical SFE. Hence, the analyst usually selects extraction conditions on an empirical basis, particularly when little is known about the sample matrix prior to extraction. In addition, the rapid demand for results in many applied analytical chemistry

situations leaves limited time to apply complex theoretical calculations to the problem at hand.

A plethora of phase equilibrium studies and attempts at modeling supercritical fluid mixtures exists in the literature.[23] These methods, unfortunately, require a considerable number of physicochemical parameters for their application to even the simplest binary solute-solvent systems. Such theories can be difficult to apply to many situations in analytical SFE due to the lack of physicochemical data on the structurally complex solutes that are frequently encountered in applied analysis problems. A review of the complex phase behavior of supercritical mixtures is given by Schneider,[24] while Johnston *et al.*[25] have produced an excellent review of theoretical attempts to model the behavior of supercritical fluid mixtures.

Despite these reservations, several attempts have been made to provide theoretical guidelines for performing analytical SFE. For example, Bartle *et al.*[26] have utilized the Peng-Robinson equation of state for predicting the solubilities of model coal tar components in CO_2. Solubilities were computed by

$$\ln s = \ln\left(\frac{P_v}{P}\right) - \ln \phi - \ln \overline{V}_l + \frac{P\overline{V}_s}{RT} \qquad (5.1)$$

where s is the solute solubility in supercritial CO_2, P_v is the vapor pressure of the solute, P is the system pressure, ϕ is the fugacity of the solute in the supercritical fluid phase, T is the system temperature, $\overline{V}_s$ is the molar volume of the solute, and $\overline{V}_l$ is the molar volume of the supercritical fluid. The Peng-Robinson equation of state was then used to calculate the fugacity of the solute in the supercritical fluid phase as

$$\ln \phi = \left(\frac{b_1}{b_2}\right)(Z - 1) - \ln\left(\frac{Z - Pb_2}{RT}\right) - \left(\frac{a_{22}}{2\sqrt{2b_2}\ RT}\right)\left(\frac{2a_{12}}{a_{22} - \frac{b_1}{b_2}}\right)\ln\left[\frac{Z + \frac{(1 + \sqrt{2})b_2 P}{RT}}{Z - \frac{(1 - \sqrt{2})b_2 P}{RT}}\right] \qquad (5.2)$$

where Z is the fluid compressibility and a and b are constants dependent on the system pressure, the critical temperature, the vapor

pressure of the solute or the fluid, and an adjustable interaction parameter. The solubilities obtained from this modestly complex equation of state were predicted to be reliable between 10 to 20% over the chosen experimental range of temperature and pressure.

Another approach that has seen wide use in predicting conditions for solubilizing solutes in supercritical fluids is the solubility parameter theory.[27] Historically, Giddings[3] has applied this concept to quantitatively describe the solvent power of dense gases according to

$$\delta_1 = 1.25\, P_c^{1/2}\left(\frac{\rho_{r,g}}{\rho_{r,l}}\right) \tag{5.3}$$

where δ_1 is the solubility parameter of the compressed gas, P_c is the fluid critical pressure, $\rho_{r,g}$ is the reduced density of the fluid, and $\rho_{r,l}$ is the reduced density of the extracting fluid in the quasi-liquid state. This concept has been further expanded by King[10] to calculate the conditions under which maximum solute solubility is realized in the extracting fluid phase. In this case, the solute-solvent interaction parameter, ψ, can be approximated by

$$\psi \approx \psi_H = \frac{\bar{V}_l(\delta_1 - \delta_2)^2}{RT} \tag{5.4}$$

where ψ_H is the enthalpic interaction parameter, δ_2 is the solubility parameter of the solute, and $\bar{V}_l$ is the molar volume of the supercritical fluid. The respective solubility parameters and $\bar{V}_l$ are functions of temperature and pressure, thereby, making ψ a function of these thermodynamic variables. Plots of ψ *vs* pressure show a minimum at a pressure corresponding to the maximum solubility of the solute in the supercritical fluid. The above approach, which combines the regular solution concept with Flory-Huggins theory, can also be used to predict the pressures required for solute miscibility with the supercritical fluid phase.[10]

Mass transfer in supercritical fluids. As noted previously, recorded solute diffusion coefficients in supercritical fluids have values between those attained in gaseous or liquid solvents. Although such data as a function of pressure are relatively scarce, solute diffusion coefficients tend to exhibit similar trends as recorded self-diffusivities of the extracting fluid. The variance of the self-

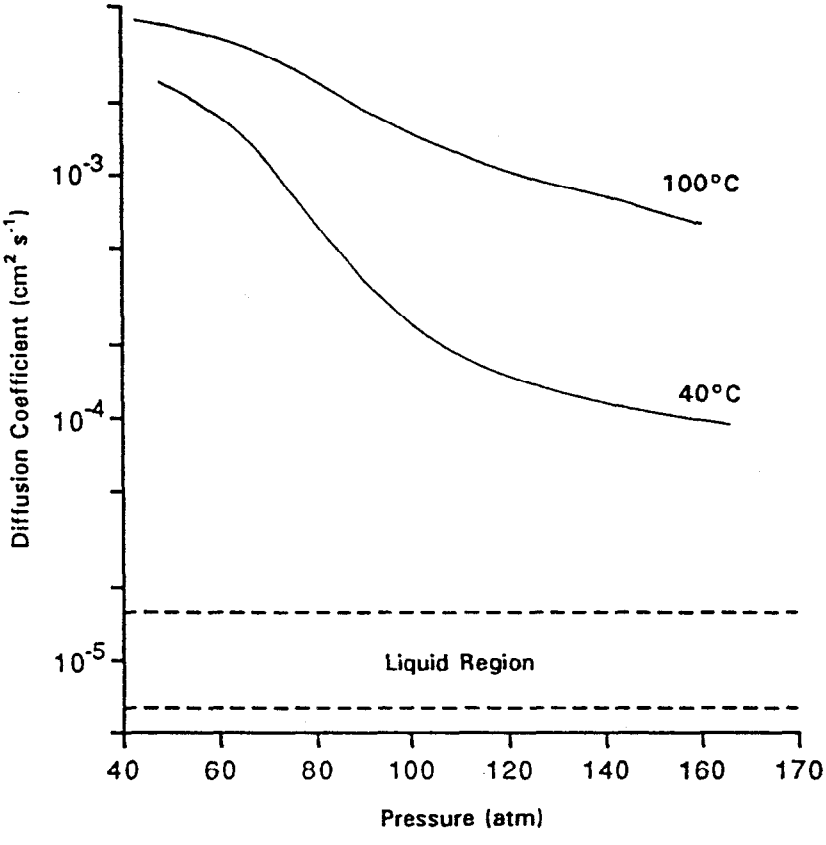

Figure 5.4. Self-diffusion coefficient of CO_2 as a function of pressure.

diffusion coefficient of CO_2 with pressure is shown in Figure 5.4, and it is compared to the range of values normally associated with liquid solvents. Note that at only modest pressures above the critical pressure for CO_2, the self-diffusivity for supercritical CO_2 is almost two orders of magnitude greater than the diffusivities recorded for the liquid state.

As the temperature of the supercritical CO_2 phase is lowered, the diffusivities begin to approach those associated with the liquid state; however, even at 40°C, the self-diffusivity of CO_2 is one order of magnitude greater than values for liquid CO_2. This trend suggests that solute interphase mass transfer rates in supercritical CO_2 will be significantly higher than those recorded in a liquefied gas. This observation has been verified by Krichevskii *et al.*[28] for the rate of naphthalene dissolution into both liquid and supercritical CO_2, and

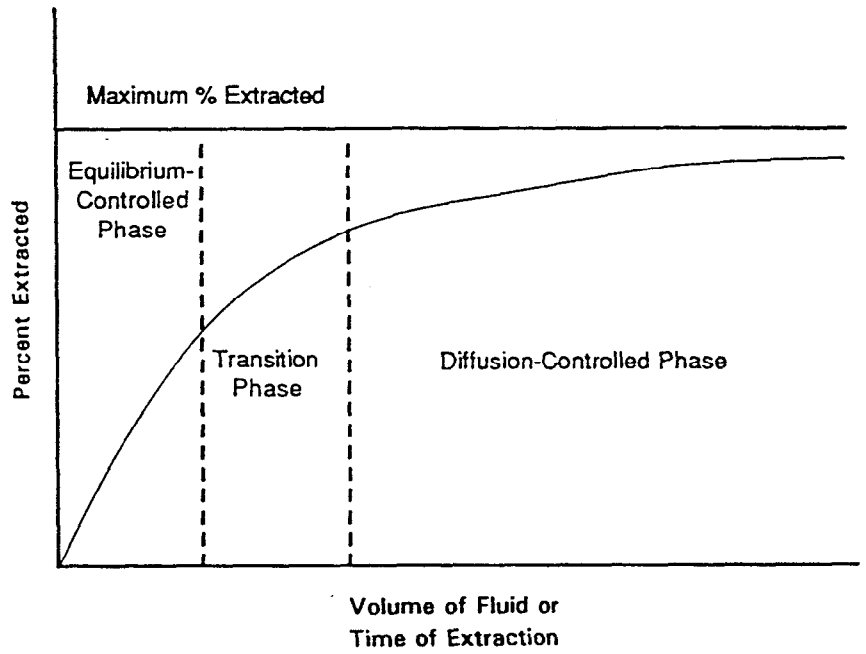

Figure 5.5. Generalized extraction curve of percent solute extracted as a function of volume of extraction fluid or time of extraction.

it accounts for the long extraction times frequently associated with Soxhlet extractions using liquid CO_2.[29]

It is interesting to examine several specific cases of SFE that exhibit rate limiting phenomena. For example, the extraction of solutes from a solid matrix enclosed in a tubular vessel using a supercritical fluid closely parallels the kinetics observed in liquid extractions. As shown in Figure 5.5, the initial portion of the extraction curve is linear, indicating that quasi-equilibrium conditions are governing the partition of the solute into the mobile dense fluid phase. After a finite time, the yield curve starts to become convex with respect to the time axis as the extraction experiences a transition from equilibrium to diffusion controlled kinetics. In the final stage of the extraction, the kinetics are dominated by diffusive mechanisms which may be quite complex, depending on the morphology of the substrate being extracted. For the case of the solid substrate, factors such as the degree of comminution or swelling of the substrate can have a profound effect on the yield curve.

Mathematical models have been formulated to account for the observed kinetics associated with specific supercritical fluid extractions of various substrates. For example, Bulley *et al.*[30] have shown that the SFE extraction of triglycerides from seeds can be described by differential equations giving the material balances in both the fluid and solid phases. The modeling of mass transfer effects for the SFE of organic solutes from water and the extraction of caffeine from coffee have been treated by Brunner.[31] Theoretical modeling for the SFE of a diverse number of substrates has been reported, including mushroom plugs,[32] mackerel powder,[33] and porous rods.[34]

Swanson *et al.*[35] have applied mass transfer theory for the case of extraction under turbulent flow conditions in a packed extractor cell. In this case, the concentration gradient is not known, so a modified mass transfer coefficient (K'_A) was utilized, defined as

$$K'_A = K_c (c_{A,i} - c_{A,f}) \tag{5.5}$$

where K_c is the mass transfer coefficient, $c_{A,i}$ is the concentration of solute A at the interface, and $c_{A,f}$ is the concentration in the adjacent bulk fluid. The factors which impact on the mass transfer coefficient, such as the fluid density or viscosity, or the geometric characteristics of the extraction cell, can be expressed as dimensionless numbers defined as

$$Sh = \frac{K_c\, d}{D_{AB}} \tag{5.6}$$

$$Re = \frac{\rho F_m}{\eta\, d} \tag{5.7}$$

$$Sc = \frac{\eta}{D_{AB}\, \rho} \tag{5.8}$$

where D_{AB} is the diffusion coefficient, ρ is the fluid density, F_m is the fluid velocity, η is the fluid viscosity, and d is the diameter of the extraction vessel. The Sherwood number can be shown to be a function of both the Reynolds and Schmidt numbers, *i.e.*, Sh = f(Re, Sc). Unfortunately, the empirical correlations that have been formulated between Sh, Re, and Sc for packed bed behavior in the

Table 5.2. Comparison of Rates of Extraction from Cells Having Two Different Diameters[a]

Diameter (mm)	Extraction (peak area counts x 10^{-3})	
	C_{18}	C_{20}
6	478	140
4	610	202

[a] CO_2, 80 atm, 70°C, 1-min extraction, equal volumes of alumina, and identical mass flow rates.

presence of dilute gases or liquids are not applicable to supercritical fluids because of large bouyant effects.[36]

The above definitions for dimensionless numbers can be used to optimize extraction conditions. For example, the Reynolds number indicates turbulence, and as the extraction cell diameter is decreased, the mass transfer within the cell should increase. The effect of the extraction cell diameter on the rate of extraction of *n*-alkanes, spiked on an alumina sorbent, is shown in Table 5.2. These samples were extracted at 80 atm and 70°C for 1 min, and subsequently cryo-trapped at -70°C and analyzed by gas chromatography. The resultant peak area counts recorded upon elution of *n*-octadecane and *n*-eicosane from the extraction cell, for equal alumina volumes and CO_2 mass flow rates, clearly show the advantages of the smaller cell diameter for enhancing extraction.

Extraction rates can also be increased when turbulence in the fluid flow pattern is increased. For example, if diffusers are placed in the ends of a 1.0-cm diameter extraction cell, the recorded extraction rate (total area counts) for the above two hydrocarbons exceeds that recorded for the 0.6-cm diameter cell in Table 5.2 under equivalent experimental conditions. Alternative methods for increasing turbulence in packed beds have been reported, including pressure pulsations[37] and ultrasonic bombardment.[38]

5.3 Off-line SFE

Off-line *vs* On-line SFE. The analyst who hopes to exploit the attractive characteristics of supercritical fluids for rapid and quantitative extraction and recovery of target analytes from bulk matrices has essentially two different approaches to choose from. SFE can be performed purely for sample preparation by collecting the extracted analytes for subsequent analysis by a variety of techniques including (but not limited to) chromatographic, spectroscopic, and gravimetric methods. This "off-line" approach is contrasted to "on-line" (or "coupled") SFE techniques in which the SFE step replaces the normal sample injection process into the chromatograph; *i.e.*, with on-line SFE, extracted analytes are transferred to and collected in a chromatographic injection loop, a thermal or sorbent trap prior to the chromatographic column, or in the stationary phase at the head of the chromatographic column itself.

Off-line SFE is inherently simpler than on-line SFE since the analyst needs only to consider the extraction and analyte collection steps. In contrast, on-line SFE requires the SFE parameters, the analyte trapping conditions, and the chromatographic separation all to be understood before the analysis can be successfully completed. A sample extracted off-line can be analyzed by any appropriate technique, and is available for multiple analyses. A sample extracted on-line, however, is dedicated to the coupled chromatographic system. Once the on-line SFE analysis is completed, the extract is no longer available for evaluation using different techniques or chromatographic parameters.

Since off-line SFE is generally simpler to perform, does not require previous understanding of necessary chromatographic conditions, and allows the extract to be analyzed by any appropriate technique, off-line SFE should be the first choice of any analyst who desires to develop SFE as a sample preparation method. The principal advantages of on-line SFE, *i.e.*, the ability to quantitatively transfer all of the extracted analytes to the chromatographic system (which results in maximum sensitivity), and the elimination of any sample handling between extraction and chromatographic separation, can be more easily exploited after the analyst has used off-line SFE to become familiar with the extraction conditions and techniques required for a particular analyte/matrix combination.

While both off-line and on-line SFE are likely to be widely used in the future, practical operational aspects should also be considered when choosing between these two general approaches to SFE.

On-line SFE requires that during the extraction (typically 10 min to 1 h), the chromatograph is not being used for what it does best, *i.e.*, performing separations. Similarly, the SFE system lies idle (except in the special case of on-line SFE/SFC, where the pumping system can be utilized for both functions) during the chromatographic separation. Automation of on-line SFE, while much discussed, is likely to be in the developmental stages for several years. Hence, when high sample throughput is desired, the choice of off-line SFE can greatly increase productivity.

The size of samples that can be extracted using SFE varies greatly depending on the goals and limitations of the extraction and analyses to be performed; however, analytical-scale SFE has generally been applied to samples ranging from 1 mg to hundreds of grams.[12,39] The size of sample used depends on a variety of factors including the size of sample needed to ensure homogeneity (*e.g.*, mg for air particulates to hundreds of grams for meat samples), the size of sample needed to achieve the desired sensitivity (which in turn depends on the percentage of the extracted analytes that can be transferred to the chromatographic system), and the difficulty in collecting sufficient quantities of the samples.

Off-line SFE accommodates large sample sizes better than on-line SFE, simply because the methods used to trap the analytes using on-line techniques require lower flows of the supercritical fluid. In contrast, on-line SFE can yield similar sensitivities as off-line SFE with much smaller samples since the potential exists with on-line techniques to transfer all of the extracted analytes quantitatively to the chromatographic column.[39] For example, on-line SFE/GC using an on-column injector for a 1-mg sample yields the same sensitivity (in terms of analyte concentration in the bulk sample) as off-line SFE of a 1-g sample when the analytes are collected in 1 mL of solvent followed by analysis using 1-μL on-column injection into the GC.

Techniques for off-line SFE. Analytical-scale SFE is typically performed using syringe pumps which are similar (or identical) to those used for SFC, although less expensive alternatives are available since SFE is normally performed at constant pressure and sophisticated pressure/density ramp controllers are not generally required. Gas compressors are also useful, particularly for larger-scale extractions where the desired flow and volume of the supercritical fluid are higher than can be conveniently provided by a syringe pump.[12] The temperature of the extraction is normally controlled by placing the extraction cell in a chromatographic oven or in a simple thermostatted tube heater.

Off-line SFE has been performed using two different modes: dynamic (in which the supercritical fluid is continuously flowing through the cell) and static (in which the cell is pressurized with supercritical fluid, and the extraction is allowed to proceed without any outflow of the supercritical fluid until extraction is finished). Both static and dynamic SFE have been used to achieve quantitative results, but dynamic extraction might be expected to yield more rapid recoveries by continuously providing pure extraction fluid to the sample. Dynamic extractions can also be performed without any valves between the extraction cell and the collection medium, since the extraction cell outlet restrictor can be placed directly in (for example) the solvent used to collect the extracted analytes. The elimination of the valve between the extraction and collection media is attractive, particularly for the extraction of trace compounds, since the chances for analyte loss or contamination are reduced. Static extractions, however, have the advantage that known modifier concentrations can be prepared by simply adding a measured volume of the liquid modifier to the cell prior to extraction.[40] Static extraction is also advantageous when large samples (*e.g.*, larger than 10 g) must be extracted, since supercritical fluid consumption and required pumping capacity can be reduced. However, the limited volume of extracting fluid may lead to incomplete extraction.

Although not as important as for SFC, the method used to depressurize the supercritical fluid as it exits the extraction cell must be carefully selected to allow the extracted analytes to be quantitatively transferred into the collection medium. Extracts from static and dynamic extractions can be depressurized through a micrometering valve,[12] or through flow restrictors made from fused-silica or metal tubing, such as restrictors used in SFC.[41] More sophisticated variable valve flow controllers have also been proposed.[42]

The method used to collect the extracted analytes is, to a large extent, dependent on the extraction fluid flow and the depressurization method. Proposed methods include depressurization of the SFE extract into a small volume of liquid solvent,[41] thermal trapping in cooled vessels,[43] and collection of the analytes onto an accumulator resin, followed by secondary extraction with liquid solvents or supercritical fluids.[42,44] The success of the trapping method depends on the total flow and volume of the supercritical fluid and its expanded gas. For example, a dynamic CO_2 extraction using a 10-cm x 30-μm i.d. restrictor will result in a supercritical fluid flow rate of *ca.* 1.0 mL min^{-1} (measured at the pump) which, when depressurized, results in a gas flow rate of *ca.* 500 mL min^{-1}.

These high gas flow rates have been found to be responsible for aerosol formation, which can result in more than 90% loss of analytes when thermal trapping is used.[43,45] However, analyte loss does not appear to be a problem when small (a few mL) volumes of liquid solvents are used to collect the extracted analytes.[41,46,47] The evaporation of the collection solvent is also much less than expected because the depressurization of the supercritical fluid cools the solvent during the extraction. Collection of the extract in a few mL of an appropriate liquid solvent is attractive also because the sample is immediately ready for analysis by a variety of chromatographic and spectroscopic techniques. Depressurization of the supercritical fluid with a micro-metering valve into a receiving vessel has been successful, particularly when large quantities of bulk matrix are extracted, as in the case of SFE of fats from meats.[12]

Applications of off-line SFE. Analytical scale off-line SFE has been applied to a wide variety of matrix/analyte combinations ranging from the extraction of bulk fats from meat products to the extraction of trace dioxins from environmental solids. While off-line SFE has been applied to both qualitative and quantitative extractions, the attainment of quantitative methodology is obviously the more important and difficult goal. Table 5.3 lists several analyte/matrix combinations for which quantitative (*i.e.*, greater than 90%) extraction and recovery of the target analytes has been achieved using off-line SFE. (The list of analyte/matrix combinations in Table 5.3 is meant to be instructive, rather than exhaustive.) Note that off-line SFE extraction times are generally less than 1 hour, while the liquid solvent extraction methods typically utilized for the same analyte/matrix combinations generally require several hours or even days to perform. In general, SFE required the use of either no liquid solvent, or only a few milliliters for sample collection (often representing a considerable advantage in solvent purchase and disposal costs). For samples with trace analytes, the concentration steps which are normally required following liquid solvent extraction were eliminated or reduced to only a few minutes. Note also that, in spite of the fact that CO_2 has been the most popular supercritical fluid for SFE, many of the studies listed in Table 5.3 have demonstrated that either N_2O or modified CO_2 (*e.g.*, with 2-10% methanol) yields greater recovery of moderately to highly polar analytes than pure CO_2. N_2O appears to have particular advantages over CO_2 when analytes such as PAHs and dioxins are extracted from sorptive matrices including air particulates and fly ash. Although not shown in Table 5.3, the ability to alter the solvent

Table 5.3. Representative Applications of Off-line SFE for Quantitative Extraction

Extracted analytes	Sample matrix	Supercritical fluid	Extraction time (min)	Refs.
Biological samples				
Fat, pesticides	Meat, sausage	CO_2	30-60	10,12
Vitamin K_1	Powdered infant formulas	CO_2	15	48
Menadione (vitamin K_3)	Animal feed	CO_2	20	49
Terpenes, aldehydes, esters, alcohols	Lemon peel	CO_2	20-30	50
Environmental and other solids				
Diuron, linuron pesticides	Soil	CO_2/MeOH, CO_2/EtOH	35-50	40
Triazine and other pesticides	Soil, humic acid, vegetables	MeOH	120	51
PAHs, PCBs	Soil, fly ash, sediment, air & diesel particulates	CO_2, N_2O, CO_2/MeOH, N_2O/MeOH, ethane	1-60	41,43,46 47,52,53
Chlorinated dibenzodioxins	Sediment	CO_2/MeOH		54
Anthraquinone	Paper, plywood sawdust	CO_2	20	55
Oil hydrocarbons	Sedimentary rocks	CO_2	15-30	56
Sorbent resins and polymers				
PAHs, O-, S-, N-PACs, alkanes, PCBs	Polyurethane foam	CO_2, CO_2/MeOH	10-30	43,57
PAHs, O-, N-PACs	XAD-2	Isobutane, CO_2/MeOH	30-45	43
Pesticides, PAHs	Tenax	CO_2	15	47,58
Polymer additives	Polyethylene	CO_2	120	59
Ionic surfactants	Reactor sludges, soil	CO_2/MeOH	30	60

Table 5.4. Comparison of Off-line and Conventional Techniques for the Quantitation of PAHs in Urban Dust (SRM 1649)

	Conventional[a]	Off-line SFE
Sample size	1 g	20 mg
Liquid solvent required	450 mL	3 mL
Bench space (for sample preparation)	5 m	1 m
Supply cost per extraction	$10	$0.5
Extraction time	48 h	1 h
Extract concentration time	3 h	0-10 min
Shortest possible total analysis time (one sample)	2 days	2 h

[a]Estimates are from S.A. Wise, NIST, and are adapted from from Ref. 62.

strength of a supercritical fluid by simply changing the extraction pressure (and/or temperature) has also been exploited to achieve class-selective extractions from complex matrices ranging from the sequential extraction of alkanes and PAHs from diesel exhaust particulates, to the selective extraction of pesticides from meat fats.[10,47,61]

The practical advantages of SFE are further demonstrated in Tables 5.4 and 5.5 by a comparison of conventional methods with off-line SFE for the extraction and recovery of PAHs from urban air particulates (National Institute of Standards and Technology, NIST, Standard Reference Material 1649). Note that SFE yielded excellent agreement with the PAH concentrations certified by NIST, and that SFE reduced the extraction time and the total time required for the analysis by two orders of magnitude.

Off-line SFE has been demonstrated by several investigators to yield quantitative extraction and recovery of a broad variety of analytes from an equally broad range of sample matrices. Studies of the quantitative results reported in the literature indicate that, as a rule-of-thumb, trace organics that are amenable to analysis by gas

Table 5.5. Quantitation of PAHs from Urban Air Particulates (NIST SRM 1649)

PAH	PAH concentration ($\mu g\ g^{-1}$)			
	Certified value[a]	Off-line SFE[b]	Split SFE/GC[c]	On-column SFE/GC[d]
Fluoranthene	7.1±0.5	8.0±0.6	7.2±0.9	7.3±1.0
Benz[*a*]anthracene	2.6±0.3	2.9±0.5	2.6±0.8	2.6±0.8
Benzo[*a*]pyrene	2.9±0.5	3.2±0.3	2.7±0.4	2.8±0.5
Benzo[*ghi*]perylene	4.5±1.1	4.4±0.3	3.9±1.0	3.6±0.9
Indeno[1,2,3-*cd*]pyrene	3.3±0.5	3.1±0.2	3.4±0.6	3.0±0.5

[a]Value certified by the NIST based on 48-h Soxhlet extraction of a 1-g sample.

[b]Based on triplicate 60-min extractions of 20-mg samples at 350 atm with N_2O/5% MeOH. Values were adapted from Ref. 41.

[c]Based on 30-min extractions of 50-mg samples at 375 atm and 50°C with CO_2. Values were adapted from Ref. 63.

[d]Based on four replicate analyses of 2-mg samples using SFE/GC/MS with supercritical N_2O. Each extraction was performed at 350 atm and 45°C. Results are adapted from Ref. 39.

chromatography can be quantitatively extracted with neat supercritical CO_2 or N_2O, although N_2O appears to have advantages with sorptive matrices. As the polarity and molecular mass of the analytes increase, more polar supercritical fluids are required to obtain quantitative extraction, and the use of polarity modifiers becomes attractive. The simplicity and low cost of performing off-line SFE, as well as the ability to analyze resultant extracts by any appropriate method indicate that off-line SFE should be the choice of the analyst for initial methods development. After extraction conditions are developed and understood using off-line techniques, the analyst can better consider the relative advantages of off-line and on-line SFE for routine analyses.

5.4 On-line (Coupled) SFE/GC

Introduction. On-line SFE/GC has several potential advantages over conventional extraction/GC analysis methods for the qualitative and quantitative determination of minor and trace analytes in

complex matrices. Quantitative extractions can often be achieved in a few minutes, and the ability to directly transfer the extracted analytes to the capillary GC reduces the potential for analyte loss and degradation, as well as eliminates sample handling steps between the extraction and chromatographic analysis. SFE/GC is particularly attractive when only limited quantities of samples are available and maximum sensitivity is desired, since, with appropriate coupling techniques, every extracted analyte molecule can be transferred into the GC column for separation and detection. With coupled SFE/GC, an entire analysis, including extraction, concentration, and gas chromatographic separation, can be completed in less than one hour. Since most SFE/GC developments have focused on open tubular column GC, the following discussions all refer to SFE coupled with open tubular GC columns.

Techniques for SFE/GC. The essential steps for performing on-line SFE/GC include supercritical fluid extraction, depressurization and venting of the extraction fluid with the associated collection and focusing of the extracted analytes, transferring the analytes to the GC column, and GC separation. Analogous to off-line SFE, the depressurization step is normally accomplished using a length of 5 to 30 μm i.d. fused silica tubing, or in some cases, crimped stainless steel tubing. Not surprisingly, the supercritical fluids used for SFE/GC are gases at ambient conditions, with CO_2 and, to a lesser extent, N_2O being the most popular. Since the supercritical fluids are converted to the gas phase for collection of the extracted analytes before GC separations can be performed, the relationship between the volume of the supercritical fluid needed for quantitative extraction, and the volume of the gas flow that results upon depressurization must be carefully considered for proper design of the SFE/GC system. For example, an extraction that uses 1 mL min^{-1} of supercritical CO_2 requires the analytes to be collected from a flowing gas stream of approximately 500 mL min^{-1}. Optimizing the relationship between the flow rate of the supercritical fluid needed to obtain quantitative extraction in a reasonable time, and the ability to quantitatively collect and focus the extracted analytes for subsequent GC analysis, is the fundamental problem to be solved by any proposed SFE/GC technique.

The SFE/GC techniques that have been reported vary primarily in the method used to collect and focus the analytes upon depressurization, and the associated procedure used to transfer the analytes to the GC column.[39,44,45,62-71] These approaches can be roughly divided into two categories: methods in which the analytes

are collected in an accumulating device external to the GC, and methods utilizing the GC for collection. External collections of the extracted analytes have been accomplished by depressurizing the SFE effluent into a cold trap placed prior to the GC.[44,45,65] After extraction, the analytes are transferred to the GC column by heating the trap and sweeping with a carrier gas. SFE/GC methods that have utilized the GC as the trapping system include depressurizing the SFE effluent directly into a retention gap at the head of the GC column,[67,68] depositing the extracted analytes directly inside the GC column[39,47,62] with the use of a conventional on-column injector, and depressurizing the SFE effluent directly inside a split/splitless injection port.[63,69-71] The addition of a switching valve between the extraction cell and the GC has also been used to allow specific fractions of the extract to be selectively transferred to the GC.[63,67] The results from each of these approaches are discussed below.

Comparison of SFE/GC Methods. The use of cold traps placed external to the GC for the collection of the extracted analytes is attractive since the supercritical fluid does not have to be vented through (or into) the gas chromatographic system during the extraction, and any potential effect of the supercritical fluid (and modifiers) on the chromatographic column and detector can be ignored.[44,45,65] Unfortunately, this approach has been limited by the efficiencies of the cold trapping systems that have been employed, and quantitative collection of the extracted analytes has not been demonstrated. Since GC analysis is aimed at relatively volatile components, the use of cold traps may have limited utility compared to their potential applications for SFE/SFC and SFE/HPLC. However, cold traps containing a suitable sorbent (*e.g.*, Tenax) that can be thermally desorbed to recover the analytes may be suitable for increasing the range of analytes which can be quantitatively collected from the depressurized SFE effluent.

Methods utilizing the chromatographic column for collecting and focusing the extracted analytes have been more successful in achieving reproducible and quantitative SFE/GC results. These techniques are divided into "on-column" SFE/GC (where the SFE effluent is depressurized directly inside the open tubular GC column and all of the extracted analytes are deposited directly in the column stationary phase[39,47,62]) and "split" SFE/GC (where the depressurization occurs inside a conventional heated split GC injector[63,69-71]). Both techniques utilize the stationary phase and cooling to collect and focus the extracted analytes during the SFE step. These approaches allow the quantitative trapping of analytes

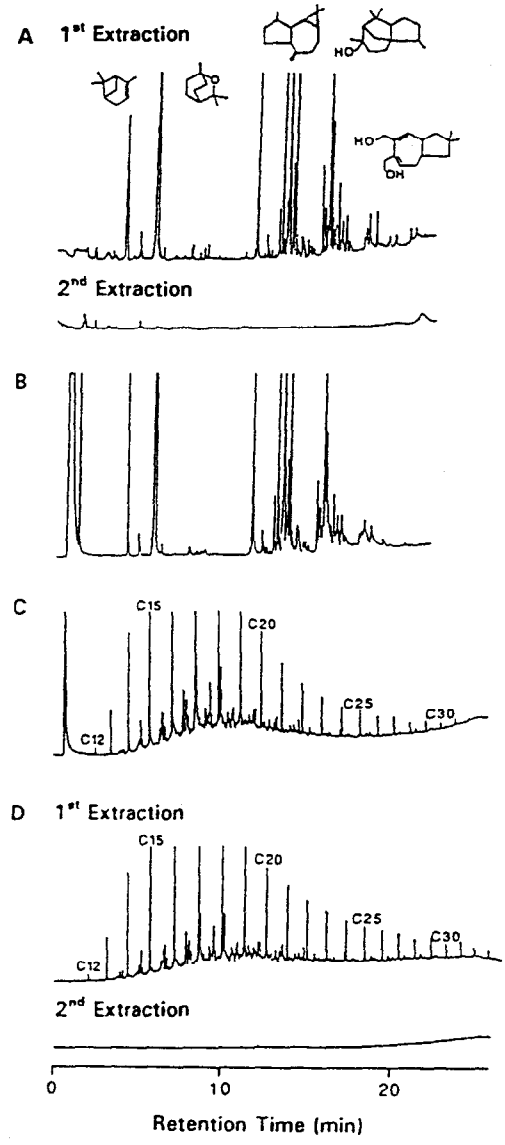

Figure 5.6. Comparisons of gas chromatograms generated using coupled SFE/GC/FID and conventional liquid solvent injections: Eucalyptus leaves analyzed by (A) on-column SFE/GC (10-min extraction with 380 atm CO_2 at 45°C) and (B) 1-μL on-column injection of a methylene chloride extract (reprinted with permission from Ref. 62); fuel-contaminated sediment analyzed by (C) split SFE/GC with a 10-min CO_2 extraction at 400 atm and 50°C, and (D) 1-μL split injection of a methylene chloride extract (reprinted with permission from Refs. 62 and 71).

less volatile than *n*-heptane and the monoterpenes,[62] and *n*-octane and benzene,[63] for on-column and split SFE/GC, respectively. As demonstrated in Figure 5.6, both techniques also yield chromatographic peak shapes that compare favorably with those obtained using conventional on-column and split injections of liquid solvent extracts.

A comparison of on-column SFE/GC with split SFE/GC is similar in many respects to a comparison between conventional GC on-column and split injections. With on-column SFE/GC, all of the extracted analytes are transferred into the GC column for analysis. This results in maximum sensitivity (*i.e.*, ppb detection limits with mg samples using conventional GC detectors); however, it also results in all of the extractable species being transferred into the column stationary phase. As with on-column injection of liquid solvents, this characteristic makes on-column SFE/GC unsuitable for samples that have high concentrations of extractable (but nonchromatographable) matrix components. In contrast, in split SFE/GC, only a fraction of the extracted analytes are transferred to the GC column (depending on the split ratio), but many of the nonvolatile matrix components are trapped on the injection port liner rather than being introduced directly into the stationary phase. With on-column SFE/GC, the analytes are never exposed to high injection port temperatures as they are in split SFE/GC. However, some matrices which contain a few percent or higher concentrations of water cannot be analyzed using on-column SFE/GC, because the water occasionally freezes in the restrictor outlet (or in the GC column) and prevents further transfer of the analytes from the extraction cell into the GC column.[62] Split SFE/GC appears to work well with wet samples since the additional heat supplied to the extraction cell restrictor by the injection port prevents freezing and subsequent plugging. Figure 5.7 demonstrates the split SFE/GC analysis of a wet polyurethane foam (PUF) sorbent plug which had been used for the solid phase extraction of organic pollutants from a coal gasification wastewater.[71] Split SFE/GC also has potential for extractions using polar modifiers as demonstrated in Figure 5.8 by the SFE/GC analysis of a solid hydrocarbon waste using formic acid-modified CO_2.[63]

As discussed above, the coupling technique used for SFE/GC determines the maximum flow rate of the supercritical fluid through the extraction cell which, in turn, affects the size of sample that can be extracted in a reasonable time. With on-column SFE/GC, the maximum flow rate that yields efficient focusing of the analytes is *ca.* 350 μL min^{-1} (for a 320-μm i.d. GC column). Assuming that a typical sample has a void fraction of 1/3 (*i.e.*, a minimum of three cell

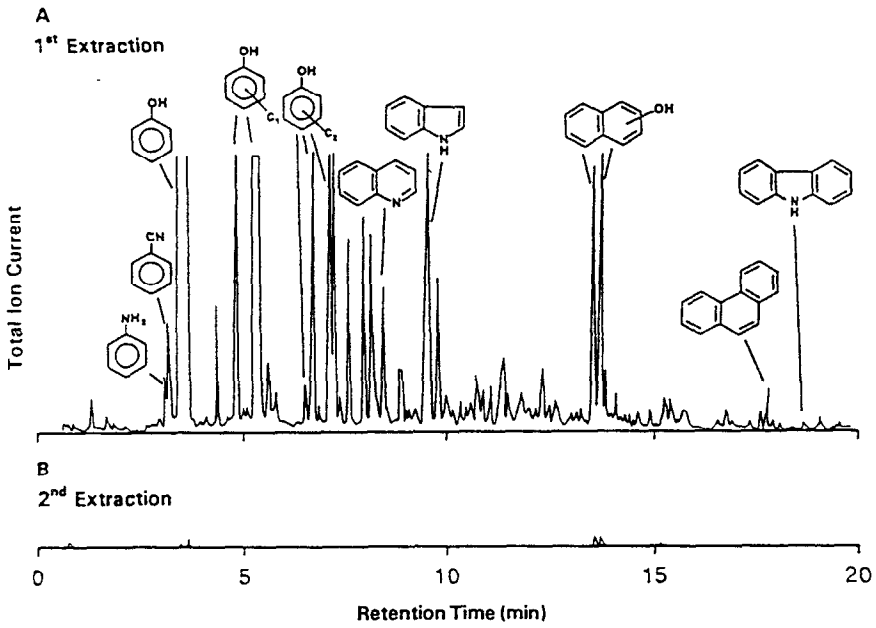

Figure 5.7. SFE/GC/MS analysis of a wet polyurethane foam (PUF) sorbent plug. Conditions: the plug had been soaked in 15 mL of a coal gasification wastewater, gently squeezed (but not dried), and extracted with CO_2 at 400 atm and 45°C. The GC oven was held at 5°C during the 10-min extraction, then the oven was rapidly heated to 70°C, followed by an 8°C min^{-1} temperature ramp to 320°C. Separations were performed with a 20-m x 250-μm i.d. open tubular column with poly(5% phenyl)methylsiloxane stationary phase (0.25-μm film thickness). The absence of significant peaks from the second SFE/GC analysis (B) indicates that the first 10-min extraction was sufficient to quantitatively recover the analytes from the PUF plug (reprinted with permission from Ref. 71).

volumes of supercritical fluid are needed for quantitative extraction) and that the extraction should be completed in 30 min, then on-column SFE/GC should be capable of extracting a sample no larger than 10 mL.[62] The fluid flow rates that can be accommodated using split SFE/GC should be substantially higher than for on-column SFE/GC.

Applications of on-line SFE/GC. The ability of both split and on-column SFE/GC to perform quantitative analysis of trace analytes from complex matrices has been demonstrated for a number of samples ranging from environmental solids to flavor and fragrance

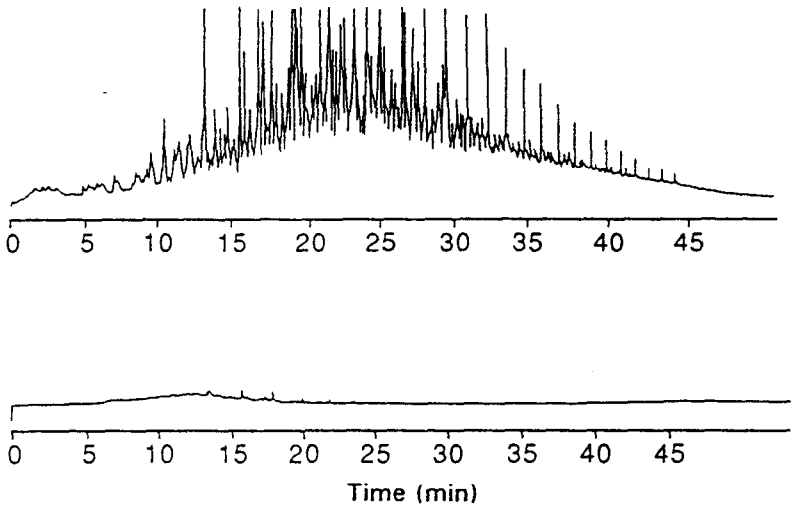

Figure 5.8. Split SFE/GC/FID analysis of a solid hydrocarbon waste (A) using CO_2/0.3% formic acid at 375 atm. The lack of significant peaks in the second extract (B) indicates that the first 30-min extraction was quantitative (reprinted with permission from Ref. 63).

compounds.[39,47,62,63,69-73] Table 5.5 shows a comparison of conventional liquid solvent Soxhlet extraction, off-line SFE, split SFE/GC, and on-column SFE/GC for the determination of PAHs on urban air particulates (NIST Standard Reference Material 1649). Note that even though the SFE/GC analyses were performed independently by two different investigators using different supercritical fluids, and using different SFE/GC approaches, the results of both SFE/GC techniques gave excellent agreement with each other and with the certified values (based on a 48-h Soxhlet extraction). Note also that the conventional method required 1 g of the particulate matter and 3 days for sample extraction and concentration (see Table 5.4). In contrast, split SFE/GC and on-column SFE/GC required only 50 mg and 2 mg of sample, respectively, and only 30 min and 15 min, respectively, for the sample extraction and concentration steps to be completed.

A comparison of the reproducibility obtained using SFE/GC with that obtained using conventional GC injections of liquid solvents is shown in Table 5.6. Split SFE/GC analysis of hydrocarbon standards extracted from alumina is compared to a split autosampler injection of the same quantity of standards in a liquid solvent.[63] On-column SFE/GC of replicate 1-mg samples of basil spice is compared to

Table 5.6. Comparison of GC/FID Peak Area Reproducibility Obtained Using Split SFE/GC, On-column SFE/GC, and Conventional GC Injections

Split SFE/GC *vs* split GC (alkane/aromatic test mixture)			On-column SFE/GC *vs* on-column GC (basil spice)		
	% RSD[a]			% RSD[b]	
Compound	SFE/GC	GC	Compound	SFE/GC	GC
Benzene	3.5	1.2	1,8-Cineole	17	11
Toluene	2.1	1.8	$C_{10}H_{18}O$ isomer	11	10
Octane	4.4	2.2	Estragole	9	11
Decane	1.4	2.5	Eugenol	17	10
Naphthalene	3.8	2.8	$C_{15}H_{24}$ isomer	9	9
Dodecane	3.1	3.3	$C_{15}H_{24}$ isomer	6	10
Pentadecane	1.8	5.2	$C_{15}H_{24}$ isomer	12	10
Phenanthrene	1.5	4.3	$C_{15}H_{24}$ isomer	6	10
Pyrene	2.2	3.4	Beta-selinene	9	15
Chrysene	5.4	3.4	$C_{15}H_{24}$ isomer	8	10

[a]Relative standard deviations (% RSD) for raw peak areas are given for 5 replicate SFE/GC analyses of the standards spiked on alumina, and 5 replicate split autosampler injections (adapted from Ref. 63).

[b]Relative standard deviations (% RSD) for raw peak areas are given for 4 replicate SFE/GC analyses of 4 different 1-mg samples of basil, and for 4 replicate 1-μL on-column injections of a methylene chloride extract of a 1-g sample of basil (adapted from Ref. 62).

conventional 1-μL on-column injections of a methylene chloride extract of basil.[62] As is shown in Table 5.6, both split and on-column SFE/GC yield reproducibilities similar to conventional liquid solvent injections. It should also be noted that the deviations shown for the on-column SFE/GC analysis of basil include the effects of sample inhomogeneity in the 1-mg samples as well as any irreproducibility in the SFE/GC technique itself.

Although on-line SFE/GC is in the early stages of development, a fairly broad range of applications has been reported, as demonstrated in Table 5.7. While many of the SFE/GC analyses are qualitative, an increasing number of quantitative results are being reported, including the analysis of certified standard reference materials,[39,63,71] the recovery of spikes, and the demonstration of

Table 5.7. Representative Applications of On-line SFE/GC

Sample matrix	Analytes	Refs.
Environmental solids		
Air and exhaust particulates	PAHs, alkanes, O-PACs, S-PACs	39,62,63,67,73
Wood smoke particulates	Guaiacol and syringol derivatives	73
Cigarette smoke particulates	Phenols, nicotine, N-heterocycles	39
Soil and sediments	PCBs, PAHs, fuel hydrocarbons	39,45,71
Sorbent resins		
Tenax:		
Vehicle exhaust, spikes	Alkanes, benzenes, PAHs, PCBs	47,66
Polyurethane foam (PUF):		
Air pollutants (exhaust, roofing tar, wood smoke)	Alkanes, benzenes, PAHs, phenols, S-PACs, guaiacol and syringol derivatives	57,62
Cigarette smoke volatiles	Phenols, N-heterocycles	57
Coal gasifier wastewater	Phenols, N-heterocycles, hydrocarbons	71
Charcoal	Hydrocarbons	44
Silica, alumina (columns)	Fuel hydrocarbons	63,69,70
Food products		
Chewing gum	Terpenes, menthol flavors	72
Citrus fruit peels	Terpenes, aldehydes, alcohols	62,72
Tobacco	Nicotine, menthol	73
Spices (*e.g.*, basil, mint, sage, rosemary, cinnamon, chili powder)	Terpenes, aromatic and aliphatic alcohols, esters sesquiterpenes, oxy-sesquiterpenes, cinnamaldehyde, coumarin	62,72,73
Miscellaneous		
Coal	Sesquiterpenes, biological markers	73
Treated utility pole	PAHs, O-, S-, N-PACs	73
Conifer tree and bush needles	Terpenes, camphor, bornyl acetate	72
Aromatic cedar wood	Cedrene, cedrol	72
Eucalyptus tree leaves	Terpenes, oxy-terpenes, sesquiterpenes	52

quantitative extraction by performing multiple extractions of a single sample.[39,57,62,63,69-73]

The development and application of on-line SFE/GC techniques is in its infancy, and any definitive statement of the potential abilities of various approaches to SFE/GC coupling would be premature. However, of the SFE/GC techniques presently available, approaches utilizing the GC column for collection and focusing of the extracted analytes appear to have significant advantages over techniques utilizing cold-trapping units external to the GC, particularly for more volatile analytes and if quantitative results are desired. On-column SFE/GC has advantages when maximum sensitivity from small samples is desired, while split SFE/GC appears to be more useful for larger and more concentrated samples. Split SFE/GC also has greater potential for the analysis of samples containing large amounts of water and samples requiring the use of polar modifiers to obtain quantitative extraction.[63,71]

The potential for performing class-selective SFE/GC by sequentially extracting samples using different pressures (or fluids) has also been demonstrated by the fractionation of PAHs and alkanes.[67,73] The ability to perform SFE/GC (or more properly, SFC/GC) analysis of different classes of analytes that are selectively eluted from sorbents has been demonstrated by the class-selective determinations of saturate, unsaturate, and aromatic fractions from fuel samples.[63] Future development of such class-selective SFE/GC techniques could be extremely useful since the potential exists to perform quantitative analysis of selected compound classes including extraction, class-fractionation, and chromatographic analysis in a total time of less than 1 h per sample.

5.5 On-line (Coupled) SFE/SFC

A number of unique devices have been developed to perform on-line SFE where the technique is coupled to SFC. In the on-line mode, SFE requires a source of compressed extraction fluid, a high pressure extraction cartridge or cell, and frequently a focusing device for concentrating the extracted solutes prior to SFC.

SFE/SFC configurations. On-line SFE/SFC equipment can range from relatively simple devices to complex arrangements involving multiple pumps, valves, and ovens. A simple approach to conducting SFE on-line in a static extraction mode is shown in

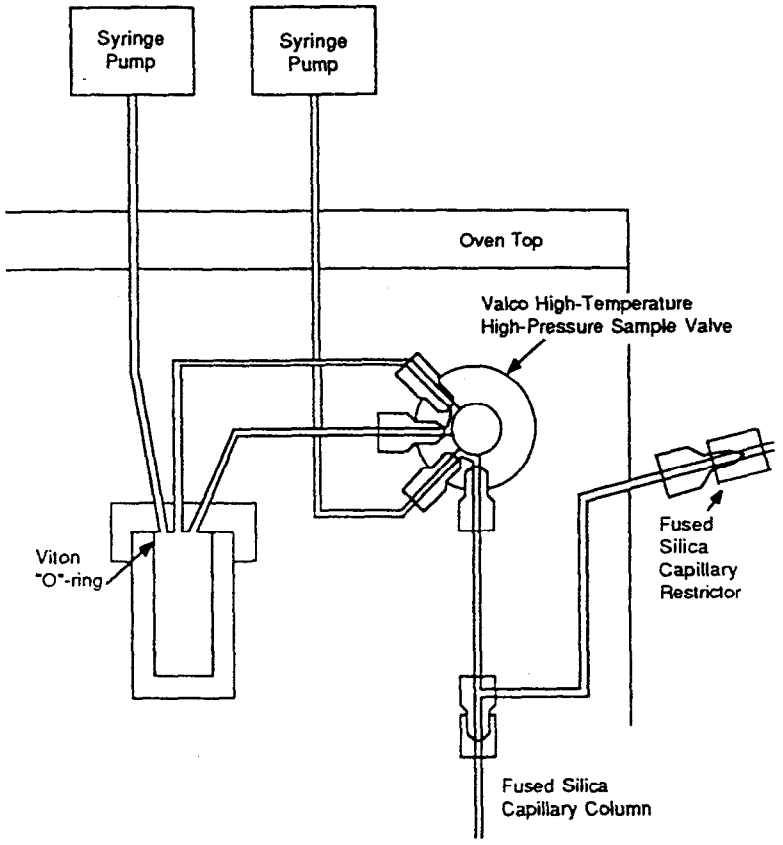

Figure 5.9. Schematic diagram of a supercritical fluid extractor/injector (reprinted with permission from Ref. 74).

Figure 5.9 where two pumps are employed: one for extracting the sample from the cell and the other for performing SFC. The sample cell in this case was a 1-cm^3 stainless steel vial sealed by a Viton "o-ring" closure. A high temperature sample valve was arranged downstream from the sample cell to permit the solute-laden extraction fluid to be introduced into the fused silica open tubular column equipped with a splitter assembly. In this arrangement, there was no focusing of the extract before SFC.

This early attempt[74] at coupling SFE with SFC deserves some further comment. The choice of material for closure seals can be critical for SFE and for the lifetime or replacement of system

components. For example, Viton o-rings will eventually blister when exposed repeatedly to high pressure CO_2,[75] and alternative materials such as nitrile-containing polymers and Teflon will result in longer service lifetimes. Similar considerations apply in the case of switching valves where seal components can be adversely affected by high temperatures. Interestingly, the extraction device shown in Figure 5.9 appears to be adequately temperature controlled with respect to the transport lines between the extractor module and the chromatography column. Improper temperature control can result in extract precipitation in the lines and valving, and can lead to failure in the SFE/SFC module. A possible problem in this regard exists when the split restrictor vent line is positioned outside the oven. Precipitation of the vented extract can occur in this narrow capillary restrictor, leading to erratic analytical results.

A dynamic extraction scheme for coupling SFE with SFC is illustrated in Figure 5.10 where the concept of an accumulator trap is utilized to focus the sample prior to SFC. Once again, a high pressure pump is utilized for the SFE, followed by the extraction cell, which is coupled to a back pressure restrictor inserted into a low dead-volume accumulator tee. During extraction, the tee is vented to atmosphere and the extract is concentrated (or accumulated) in the tee. After extraction, the valve is switched and supercritical fluid is introduced through the side arm of the tee to transfer the sample to the chromatographic column. The transfer tubing between the accumulator trap and chromatographic column is critical to obtaining good chromatographic performance. With a section of uncoated fused silica tubing as the transfer line, the analytes are unretained during transfer until they reach the analytical column where they are concentrated in the column stationary phase due to the difference in migration rates of the extracted solutes in the two sections of tubing (phase ratio focusing). Focusing between the transfer tubing and column can be improved by operating the transfer region at a temperature that is sufficient to lower the density of the supercritical fluid. This results in a reduction of the extract solubility in the supercritical fluid mobile phase and greater concentration in the stationary phase.

More sophisticated approaches for conducting SFE/SFC are possible. On-off and multiport switching valves can be arranged to permit simultaneous extraction or venting of the extraction cell and accumulator, while supplying supercritical fluid to the chromatographic column. Extracts can be focused prior to chromatography by a cryocooling tee inserted in the flow path prior to the column. Subambient temperatures can be attained at the tee

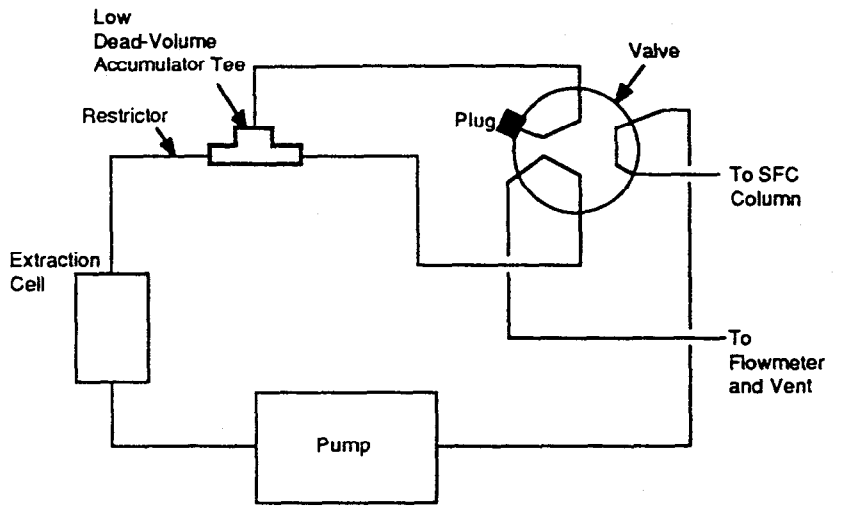

Figure 5.10. Schematic diagram of a combined supercritical fluid extractor/chromatograph utilizing a retention gap to concentrate the extract before chromatography.

by Joule-Thompson expansion of CO_2 through a micrometering valve.[46] Defocusing of the collected solutes is achieved by cessation of the external CO_2 flow and by increasing the temperature of the chromatographic oven in which the tee is located. In this arrangement, purging of the extractor cartridge can be accomplished during SFC analysis by opening a needle purge valve when a multiport valve is in the "column" position.

For on-line SFE/SFC static extractions, the sample to be extracted is held under pressure in a cell for a finite length of time before introduction into the supercritical fluid chromatograph. Aside from the ability to precisely measure fundamental parameters, such as partition coefficients of solutes in supercritical fluid media, the static method seems to have few advantages to offer the analyst. Extraction in this case is very dependent on the mass transfer kinetics of the solutes from the sample matrix. Wheeler and McNally[40] have shown that the static mode can even take days to effect complete extraction.

Recycle extraction schemes offer some interesting possibilities for SFE/SFC. One reported mode of operation[76] involved the pressurization of a recycle loop by pumping extraction fluid into the recycle loop until the desired extraction pressure had been attained for a given temperature. The recycle loop of the system was then sealed and a second pump in-line with the loop was used to circulate

a fixed volume of fluid (determined by the dimensions of the recycle loop) through the sample in an extraction cell. In this mode, intermittent sampling *via* an injection valve can be utilized to characterize the extract in the circulating fluid phase. Accurate quantitative data from such a device can only be achieved by judiciously adjusting the size of the recirculation loop relative to the anticipated level of extractables from the sample matrix for a given extraction pressure and temperature. Continuous recirculation of the extraction fluid through a sample, with subsequent precipitation at a lower temperature and pressure, is another possible recycle mode.

A novel approach to SFE/SFC is the use of a thermal modulator between the extraction cell and the chromatographic column.[77] The modulator is usually made from a short segment of a fused-silica open tubular column, which is coated on the outside with electrically conductive paint so that it can be heated rapidly by a pulse of electric current. As the supercritical fluid stream from the extraction cell flows through the modulator, solutes partition into the stationary phase in the usual manner according to the partition coefficient. When an electric pulse is applied, the solutes either desorb as a concentration pulse or absorb as a vacancy pulse because of the reduction in density, depending on the normal operating temperature of the modulator tube. The amplitude of the signal generated is proportional to the concentration of analytes flowing through the system. By performing a series of pulses and applying multiplex or correlation chromatography, selective and sensitive analysis of target analytes can be achieved. The advantage of this approach is that trapping or accumulation of the sample before the chromatographic step is not necessary, thus eliminating the most troublesome step in on-line SFE/SFC. The major limitation to this approach is that it is currently restricted to nonprogrammed SFC. While only preliminary results have been obtained using this technique, future application appears to be quite promising.

Extraction cell configuration. Extraction cells for SFE/SFC have tended to range between 150 μL and 50 mL in total volume in order to avoid overloading the open tubular and microbore columns that are commonly used in SFC. A particularly attractive source for these devices has been the guard cartridges which are utilized in high performance LC; however, care should be taken to ensure that these cells are properly rated for high pressures. Figure 5.11 illustrates common configurations which are commercially available. The use of these tubular extractors requires that attention be paid to such factors as the total amount of extraction fluid that passes

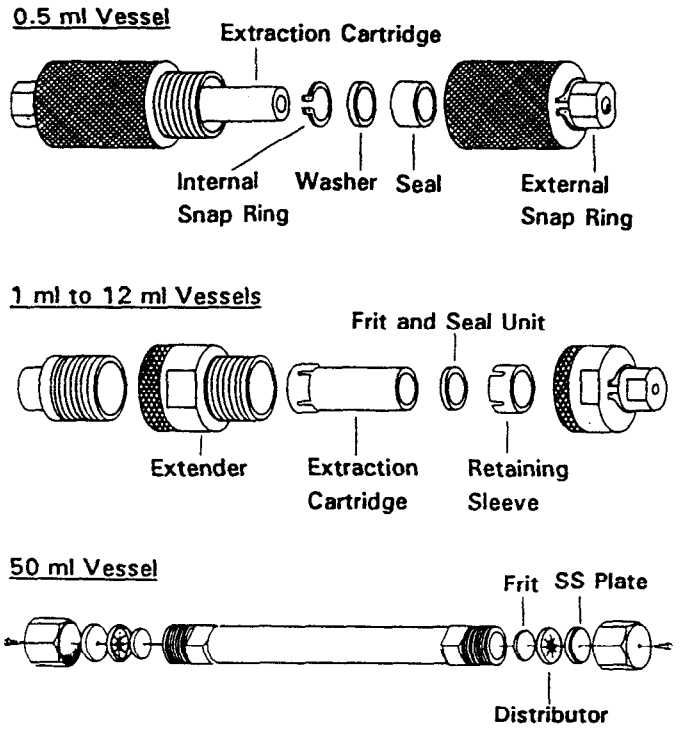

Figure 5.11. Typical extraction cells used in analytical supercritical fluid extraction.

through the cell in order to assure reproducible extraction. One can calculate the linear velocity (u) through the extraction cell using

$$u = \frac{F_a \rho_c}{A_c \rho_a} \tag{5.9}$$

where F_a is the flow rate under ambient conditions, ρ_c is the fluid density within the cell, ρ_a is the fluid density under ambient conditions, and A_c is the cross-sectional area of the extractor cell. Hence, with knowledge of the cell geometry and u, one can estimate the total number of column volumes of fluid/unit of time that pass through the extractor. The degree of sample contact with the extraction fluid is critical, and this can be increased by keeping the volume of the extractor cell small with respect to the sample volume. In fact, coupled extractor cells as small as 85 μL have been reported in the literature.[78]

Solute trapping after extraction. Trapping techniques for combined SFE/SFC are still evolving, but recent research has identified factors which are critical for optimizing the technique. For example, the effect of trapping temperature on the recovery of analytes can be understood by invoking the mass transfer coefficient[35] defined in Equation 5.5, and substituting the partial pressure for the concentration of the solute. This indicates that the difference in partial pressures is the driving force for focusing the solute. Hence, if the solute's partial pressure at the fluid/solid interface is less than its partial pressure in the supercritical fluid, then deposition will take place. Since solute vapor pressure is a function of temperature, lower temperatures should lead to increased rates of solute deposition. This effect is nicely illustrated in Figure 5.12 where the higher trap temperature leads to discrimination in the recovery of the more volatile *n*-alkane analytes.[45] Cryofocusing temperature also has a pronounced effect on peak widths in subsequent chromatography.[79] For instance, too high cryofocusing temperature will lead to peak broadening of the more volatile analytes in the extracted mixture. Conversely, too low cryotrapping temperature may lead to plugging in the restrictor leading from the extraction cell.

An alternative focusing method to thermal-based trapping is the use of a sorbent for concentrating the extracted solutes. In this case, the high surface area of the sorbent combined with the adsorptivity of the solute at the fluid/solid interface creates a favorable situation for concentrating the extract after SFE. When utilizing this method, it is important to assure that breakthrough of the analytes from the sorbent trap does not occur. King[80] has shown that the solute breakthrough volume varies with pressure in the presence of a supercritical fluid; therefore, one must select a low enough pressure or temperature to limit the mobility of the solute on the sorbent trap. Desorption from the trapping medium can be accomplished by increasing the temperature and/or by using the supercritical fluid to desorb the collected sample. The surface areas and porosities of trapping sorbents can be altered by exposure to supercritical fluids,[81] and this may ultimately limit their service lifetimes.

The use of adsorbents after SFE can also aid in the fractionation and cleanup of complex extracts that frequently result from using SFE for complex natural product mixtures. The combination of adsorbent columns with SFE is an emerging area of research, and limited work has been reported to date. Nevertheless, Campbell and Lee[61] have shown that complex petroleum- and coal-derived mixtures can be fractionated using siliceous adsorbents prior to capillary gas

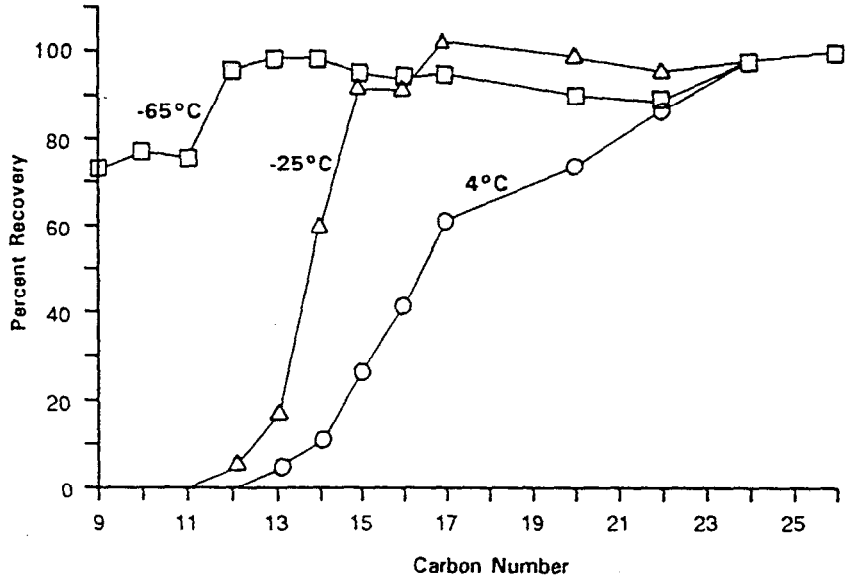

Figure 5.12. Recoveries of extracted *n*-alkanes as a function of carbon number at different cryotrapping temperatures (reprinted with permission from Ref. 45).

chromatographic characterization. Specific adsorbents, such as ion exchange resins, offer the possibility to isolate specific compounds free from interfering coextractives. This principle has been recently demonstrated by Schaeffer *et al.*[82] by successfully capturing an alkaloid on a cation exchange resin from a supercritical CO_2/ethanol fluid phase. The use of different types of supercritical fluids can also influence the desorption of analytes from adsorptive matrices.[80] Several investigators have shown that supercritical nitrous oxide is more effective than supercritical CO_2 in removing solutes from adsorbents and active solids.[39,41,83]

Liquid sample extraction. The SFE devices described above are largely designed for the extraction of solid samples. SFE of liquid samples presents a different challenge to the analyst due to the small, but finite, solubility of water in most supercritical fluid media, and the possibility of phase inversion effects as the extraction fluid is compressed. A simple method has been reported by Hedrick and Taylor[84] for the quantitative extraction of a phosphonate herbicide from aqueous media using a tubular extraction cell. In this method, the fluid is bubbled through a partially-filled cell, collected in the headspace, and routed out of the cell through an exit tube which

projects above the liquid miniscus in the extraction cell. The addition of salts and acids to the aqueous medium promoted extraction of the solute into the supercritical fluid phase, thereby reducing the extraction time from several hours to minutes. More elegant devices based on phase switching principles have been developed for affecting SFE of aqueous samples.[85,86] One of the reported methods[85] is based on the use of a phase separator designed for liquid-liquid extraction to partition the dissolved analyte from water to a compressed CO_2 phase, while the other[86] involves adsorption of the target analyte on a hydrophobic sorbent, subsequent removal of the interfering hydrophilic components by a liquid solvent, drying, and desorption into supercritical CO_2.

5.6 Optimization of Analytical SFE

Analytical SFE is currently an evolving technique in which many experimental parameters and problems are still under investigation. Optimization of SFE is, perhaps, the key area of most concern to the analyst since it impacts on the accuracy and precision that can be obtained with this technique. Some of the experimental factors which impact SFE have already been discussed, including the pressure and temperature of the extracting fluid. Concern must also be paid to the time period of extraction, fluid flow rate through the extraction cell, sample matrix effects, homogeneity of the sample, and system contamination.

The optimum extraction time is dependent on the experimental pressure and temperature as well as on the flow rate of the fluid through the extraction cell. For unknown samples, the extraction time can best be found by experimentally conducting successive extractions to determine the completeness of extraction. An example of this procedure is depicted in Figure 5.13 in which two successive extractions were conducted on an aquifer sediment sample with supercritical CO_2 under the listed conditions. In this particular case, a 3-min extraction was not sufficient to complete the extraction of the supercritical CO_2-soluble components.

The use of a nondestructive detector in tandem with SFE/SFC can also aid the analyst in determining the extent of extraction. For example, diverting the extraction fluid through a UV detector prior to chromatographic analysis would allow the extent of extraction to be monitored, provided the compounds of interest absorb light. A more elaborate technique involving the use of radioactive tracers[40,55]

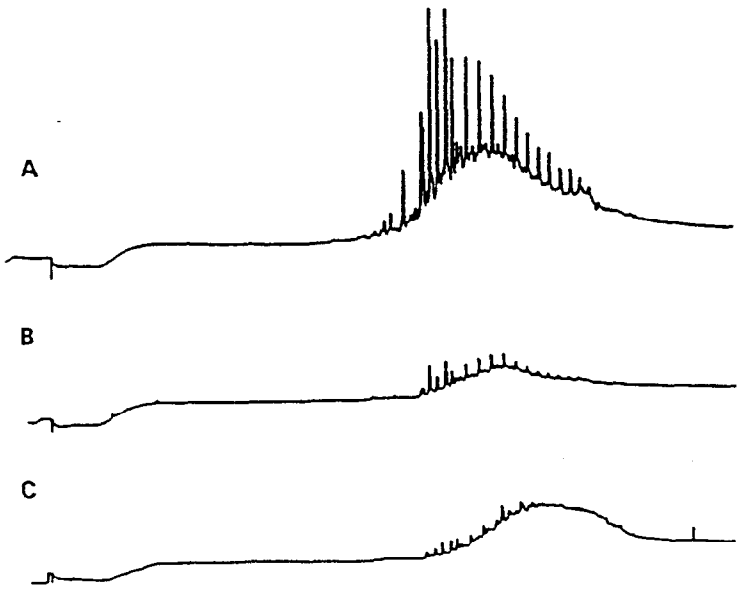

Figure 5.13. On-line SFE/SFC analysis of an aquifer sediment sample for two subsequent extractions: (A) 1st extraction, (B) 2nd extraction, and (C) blank. SFE conditions: 3 min; CO_2; 200 atm; 60°C; cryofocused extract. SFC conditions: 15-m x 50-μm i.d. open tubular column; poly(50% *n*-octyl)methylsiloxane stationary phase; CO_2; 100°C; pressure program from 50 to 300 atm at 3 atm min^{-1}; FID.

has also been employed for measuring the completeness of extraction from soil matrices. Knowledge of a principal component's solubility in the supercritical fluid can also assist the analyst in choosing the proper extraction time, since the percent recovery is a function of the distribution coefficient of the compound, the phase ratio in the extraction cell, and the number of extractions (column volumes) utilized.[87] Optimum extraction will, of course, be realized when the chosen cell volume is evenly filled with sample and there is sufficient contact between the extraction fluid and the sample.

The sample matrix can also exert a profound effect on SFE. Such factors as particle size and shape, surface area and porosity, moisture content of the sample, and level of extractable matter will impact on the analytical results. In general, a reduction in particle size will improve the extraction efficiency[46,88] and lead to a reduction in extraction time; however, deleterious effects may also occur, such as large pressure drops in the extraction cell and plugging of the extractor cell due to sample compaction. Samples having large surface areas and correspondingly high porosities may inhibit

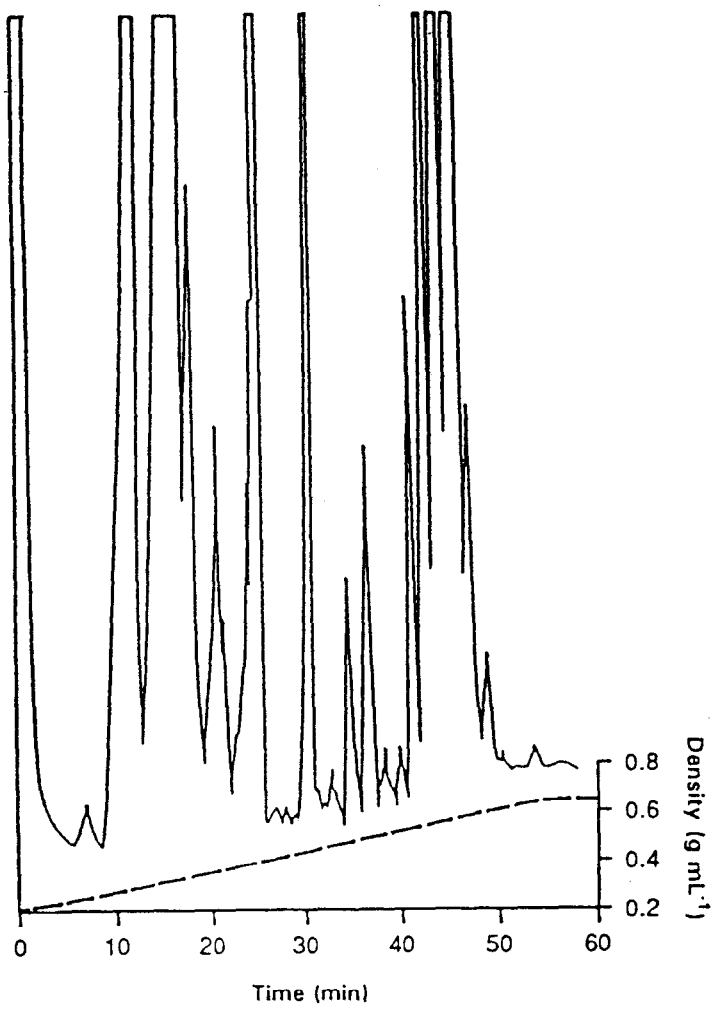

Figure 5.14. Supercritical fluid chromatogram of a supercritical fluid extract (dissolved in chloroform) of spiked poultry liver (15 ppm Bendiocarb). Conditions: 12-m x 100-μm i.d. open tubular column; poly(5% phenyl)methylsiiloxane stationary phase; CO_2; 107°C; program from 0.25 g mL^{-1} to 0.65 g mL^{-1} at 0.008 g mL^{-1} min^{-1}; FID (reprinted with permission from Ref. 95).

extraction, particularly if the sample has a heterogeneous surface with active sites. In this case, higher extraction temperatures have been shown to improve the recovery of analytes, such as pesticides from soil matrices.[40,89] Alternatively, the addition of organic modifiers to the extraction fluid may also aid in the recovery of the analytes from specific soils.[90,91] Moist samples can also present a

particularly difficult problem when using supercritical CO_2 as the extracting fluid. For the extraction of nonpolar to moderately polar solutes in matrices containing high moisture levels, it is often necessary to remove most of the water by some technique such as oven drying,[12] microwave drying, or freeze drying.[92] Alternatively, the sample can be mixed with an adsorbent such as anhydrous sodium sulfate which can adsorb water during the extraction. In certain cases, the presence of moisture in a sample matrix can result in better recoveries of trace levels of polar analytes due to enhanced solubility in the supercritical fluid phase[93] and reduction of matrix-analyte interactions.[94]

Sample matrices containing a high level of extractables can present additional problems for the analyst. The presence of a high level of coextractives from the SFE can render subsequent chromatographic analysis useless without additional class-fractionation steps. This is particularly true for lipid-rich sample matrices where such components show a propensity for partitioning into such fluids as supercritical CO_2. An example of this phenomenon is illustrated in Figure 5.14 where a poultry liver sample has been extracted with supercritical CO_2 and subsequently chromatographed using SFC. In such samples, the ability to discern a particular target analyte among the coextracted peaks is often difficult. This problem can be partially overcome by using chromatographic detectors having a specificity for the analyte of interest. For example, King[96] has shown that chlorinated pesticides in the presence of coextracted lipid matter can be selectively monitored using an electron capture detector, while Schneiderman *et al.*[48] has employed an LC electrochemical detector for detecting substances such as vitamins in infant formulations.

Specific problems exist in the field of analytical SFE which must be addressed in the future if the technique is to expand into other areas of application. Handling of the extraction cells should be done with caution since even fingerprints on the surface of the cell can produce a "chromatographic fingerprint" when the extract is analyzed by SFC. Figure 5.15 shows the results obtained when an extraction cell was handled before SFE/SFC. The peaks appearing in the profile are probably lipids from the analyst's hands. This problem is particularly enhanced when using extraction cells that have internal parts, such as ferrules or fritted disks, that must be reassembled before the commencement of extraction. Caution should also be exerted when using detergents for leak testing or cleaning of extraction cells, since it has been demonstrated that surfactants can be readily solubilized in supercritical fluid media.[97]

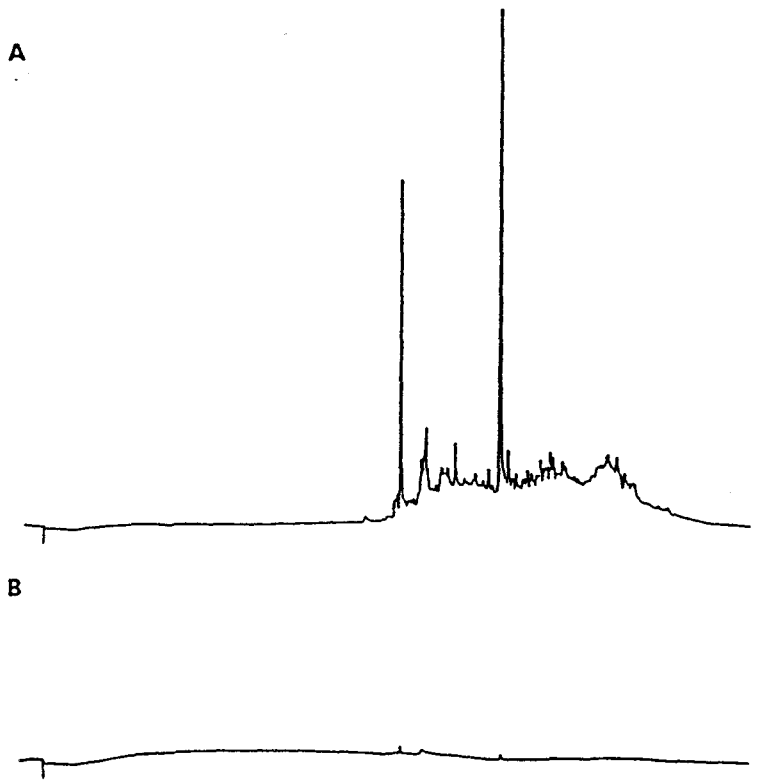

Figure 5.15. SFE/SFC profile (A) after and (B) before exposure of an extraction cell to human fingerprints. SFE conditions: 3 min; CO_2; 200 atm; 60°C; cryofocused extract. SFC conditions: 15-m x 50-μm i.d. open tubular column; poly(50% *n*-octyl)methylsiloxane stationary phase; CO_2; 100°C; pressure program from 50 atm to 300 atm at 3 atm min^{-1} after a 1-min initial isobaric period; FID.

Another vexing problem in analytical SFE is the purity level of the extraction fluid. High purity gases currently available have been purified for use in gas chromatographic trace analysis or SFC. Early analytical SFE studies revealed the need for even higher levels of gas purity because of the concentrating effect of the SFE step. This problem has been attacked by several methods: the use of adsorbent traps prior to SFE, choosing an alternative source for the extraction fluid, and by modifying the detection scheme during the chromatographic analysis. The use of activated alumina has found particular favor among many analysts for rendering CO_2 free from

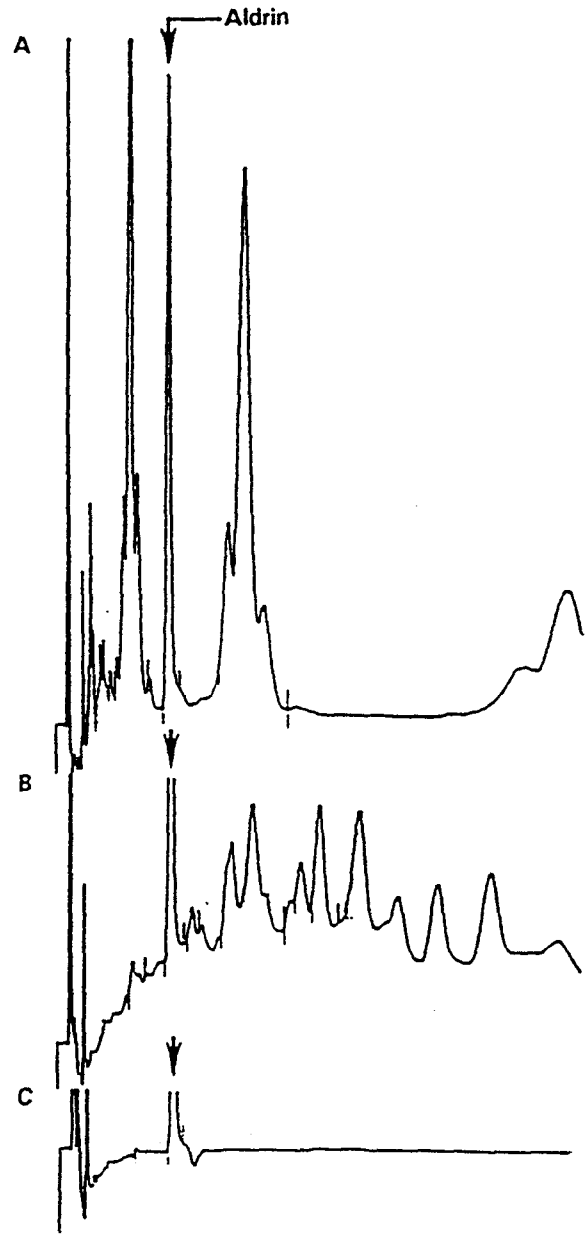

Figure 5.16. Gas chromatographic detection (ECD) of impurities in CO_2 used for SFE showing (A) Teflon contamination, (B) welding-grade CO_2, and (C) SFC-grade CO_2. SFE conditions: ~45 min; ~220 mL CO_2 (liquid); 160 atm; 40°C.

FID-detectable impurities. Food grade CO_2, derived from fermentation sources, also appears to be a good choice for minimizing contamination in the extraction fluid.[68] The use of highly sensitive element-specific detectors, such as the electron capture detector (ECD), in trace analysis creates an additional criterion in gas purity. As is shown in Figure 5.16, there is a considerable difference in the ECD-detectable impurity levels between a tank of welding grade CO_2 and the highly purified SFC-grade product. The introduction of specific materials such as elastomeric-based seals and o-rings into the flow path of the extraction fluid can result in a significant extraction of ECD-sensitive impurities into the compressed fluid. Chlorofluorohydrocarbons utilized in the cleaning of compressed gas cylinders have also been implicated as a source of impurities in fluids used for SFE.

Currently, analytical scale SFE has certain limitations that must be addressed if the technique is to find widespread acceptance in the analytical community. Certain laboratory environments (*i.e.*, regulatory laboratories) prepare samples in a parallel mode, suggesting that the development of extractor modules which allow the processing of multiple samples should be pursued. Such devices may eventually involve highly automated stream switching techniques to allow improved mating of SFE with the various forms of chromatography. The problem of optimal sample size should also be studied, since current equipment has been miniaturized in order to be consistent with the small sample size requirements of microcolumn chromatographic methods. Unfortunately, such small samples may not be representative of the overall sample and, hence, bias the analytical result. This problem is particularly amplified in trace analysis where the detection limits of many chromatographic assays are seriously challenged. The occurrence of small leaks in a microextraction system can be much more problemmatic than in larger extraction equipment and must be eliminated to obtain consistently reproducible results.

Despite the above reservations, very respectable levels of detection and precision have been achieved using analytical SFE. As tabulated in Tables 5.3 and 5.7, a wide spectrum of sample types are amenable to analytical SFE. Detectable amounts of analytes range from mg to pg quantities in such diverse matrices as urban dust, basil, and rat chow. There is a wide range in the recorded precision levels for the cited examples that is partially dependent on the nature of the analyte, the matrix being extracted, and the concentration level of the analyte. In many cases, the RSDs of SFE are as good as those of the chromatographic method used for the quantitation. The

whole field of analytical SFE will undoubtedly be improved in the near future as the technique is modified and practiced by additional analytical chemists.

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